Ac-Ile-Glu-Thr-Asp-AFC (Ac-IETD-AFC)

Cat. # G5100, G5101

Also Known as:	Ac-IETD-AFC; Caspase-8 substrate
Cas#:	211990-57-7
MW (no tag):	729.7 Da
Formula:	C31H38F3N5O12
Source:	Synthetic
Tag:	N/A
Stock Buffer:	Powder
Solubility:	Soluble in DMSO up to 10 mM (~ 7.3 mg/ml)
Concentration:	N/A
Quality Assurance:	> 95% by HPLC
Description:	Ac-IETD-AFC is a fluorogenic substrate of caspase-8, caspase-10 and granzyme B. Working concentration of this substrate is 25-50 µM. The released AFC (7-Amino-4-trifluoromethylcoumarin) fluorescence can be detected by a fluorimeter or a plate reader using excitation/emission wavelengths at 400 nm/505 nm, respectively.
Storage:	Eligible for room temperature shipping. Store at -20°C upon receiving; avoid multiple freeze-thaw cycles after dissolving in DMSO. Protect from light.

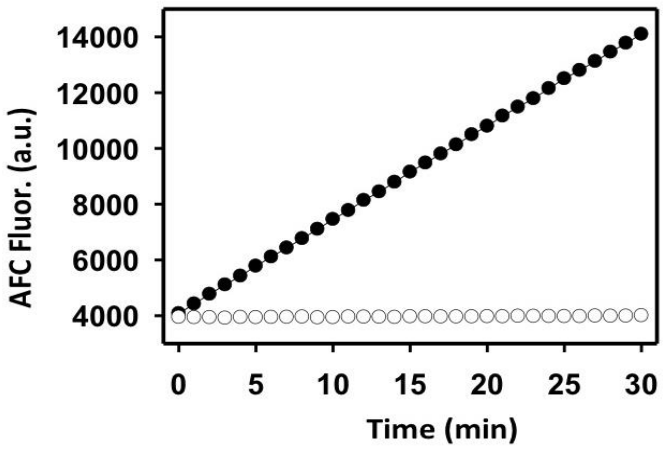
Protocol:

Users are strongly recommended to optimize conditions based on their needs.

1. Briefly spin the product packing tube using a desktop centrifuge to pellet the powder before removing the cap.
2. Prepare a 10 mM substrate stock in DMSO: add 0.685 mL DMSO to 5 mg Ac-IETD-AFC powder or 3.42 mL DMSO to 25 mg Ac-IETD-AFC powder.
3. Prepare 1X Reaction Buffer: 20 mM Tris, pH 7.6 at 4 ^{0}C, 150 mM NaCl, and 2 mM DTT.
4. Prepare 2X substrate (50 µM): add 25 µl substrate stock prepared in step 2 to 4,975 µl warmed (37 ^{0}C) 1X reaction buffer. Briefly vortex to dissolve.
5. Mix 50 µl apoptotic cell/tissule lysates (using more or less depending on capase amounts in your samples) or recombinant caspases with 50 µl 2X substrate prepared in step 4. Incubate at 37 0C for 10-60 min (users should optimize incubation time).
6. Record AFC fluorescence using a fluorometer or a plate reader using excitation/emmission wavelengths at 400 nm/505 nm, respectively.

7. Alternatively, released AFC fluorescence can be recorded continuously using a kinetic mode when the substrate is mixed with samples.
8. The reading from a control reaction should be subtracted as the background signal. An appropriate control reaction is: apoptotic cell/tissue lysate + substrate + a caspase inhibitor (such as 20 µM Z-VAD-FMK).

Image:



HCT116 cells were incubated with 50 ng/ml TRAIL for 4 hours to induce extrinsic apoptosis. 50 ug HCT116 cell lysates were incubated with (open circles) or without Z-VAD-FMK (solide circles) for 10 min at 37 ^{0}C, and then 25 uM Ac-IETD-AFC was added to initiate the reaction. AFC fluorescence was recorded with a plate reader using excitation/emission filter set at 400/508 nm, respectively.

References:

Poreba M., et al., Cold Spring Harb Perspect Bio. 2013, a008680.